AD_____

AWARD NUMBER: W81XWH-13-1-0337

TITLE: Drug Delivery for Peripheral Nerve Regeneration

PRINCIPAL INVESTIGATOR: Jayant P. Agarwal, MD

RECIPIENT: University of Utah, Salt Lake City, UT 84112

REPORT DATE: September 2014

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
September 2014	Annual	1 Sep 13-31 Aug 14

4. TITLE AND SUBTITLE
Drug Delivery for Peripheral Nerve Regeneration

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-13-1-0337

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Himanshu J. Sant and Jayant P. Agarwal

E-Mail: Himanshu.sant@utah.edu, Jay.Agarwal@hsc.utah.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Department of Surgery Research Laboratory
University of Utah
30 North 1900 East #3B400,
Salt Lake City, UT 84132

8. PERFORMING ORGANIZATION REPORT NUMBER
55900484

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Nerve injury can occur due to penetrating wounds, compression, traumatic stretch, and cold exposure. Despite prompt repair, outcomes are dismal. In this work, a polylacticcoglycolic acid (PLGA) nerve conduit with associated biodegradable drug reservoir is designed, fabricated, tested. Devices loaded with nerve growth factor (NGF) are evaluated for sustained drug release and axon growth enhancement in dorsal root ganglion (DRG) cells with the released drug.

In the first year of this 18 month project we have completed device fabrication of the nerve guide conduit and drug delivery reservoir. Work is continuing to assess device efficacy and biocompatibility in Sprague dawley rat sciatic nerve model.

15. SUBJECT TERMS nothing listed

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	22	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

1. INTRODUCTION:

Combat gear for the modern day warrior has greatly improved protection for the head and body, but limbs are still highly exposed to injury. Subsequently, the most frequent combat nerve injuries are in the upper and lower extremities. In terms of the general population and non-combat veterans peripheral nerve injuries affect 2-3% of trauma patients and vastly more subsequent to tumor extirpation or iatrogenic injury. Patients often suffer from life-long loss or functional disturbances mediated by the injured nerve, which can severely diminish their quality of life. Unfortunately, current treatments often result in inadequate or untimely repair, which can result in lifelong deficits in muscle function or sensation. Nerve injuries with large gaps (>1cm) require special bridging strategies for tension-free repair. Autologous nerve grafts serve as the state-of-the-art in repairing such gaps but numerous challenges associated with this approach results in functional benefits to only 40-50% patients. Much progress has been made in the field of artificial nerve conduits with collagen and polyglycolic acid (PGLA) conduits commercially available and in use. These hollow tubes act as axon guides for the regenerating nerves and can allow for tension free bridging without the need to harvest donor nerve. A number of research groups have proposed conjugating drugs into these conduits or using other biodegradable components such as hydrogels. The shortcomings of current devices in terms of burst effect, nonuniform dosage, and uneven drug delivery, necessitates a new approach to deliver drug for nerve regeneration. This project focuses on a novel approach to deliver drugs to a regenerating nerve in a controlled manner. This unique design consists of a biodegradable drug delivery device, capable of delivering proteins and small molecules at zero order kinetics, attached to a biodegradable PGLA conduit. The drug delivery device consists of three main components: (i) a drug reservoir, (ii) a biodegradable polymer matrix for controlled drug delivery, and (iii) a nanoporous filter for controlled drug diffusion. We will study the efficacy of our novel biodegradable nerve conduits to (1) continuously deliver small molecules or growth factors to the regenerating axons at a controlled rate and (2) improve the degree of axon regeneration and functional recovery. This project will focus on the local delivery of nerve growth factor (NGF), a protein, which has been shown to enhance peripheral nerve regeneration.

This report outlines the results of the first year of this 18 month project. We have completed optimization of PLGA conduit fabrication. Preliminary NGF bioactivity studies have been completed. While the animal studies have not been started, the necessary approvals have been obtained and the protocols have been developed.

2. KEYWORDS: Provide a brief list of keywords (limit to 20 words).

Peripheral nerve regeneration, nerve conduit, nerve growth factor, poly lactic co-glycolic acid, drug-delivering conduit, axon elongation, drug delivery device

3. ACCOMPLISHMENTS:

The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.

What were the major goals of the project?

1. Manufacture Devices for use in 15mm nerve gap .....(Gale) (months 0-5)
    a. Optimize PGLA ratios.....(Gale)(months 0-1)

b. Optimize nanoporous membrane dimensions.....(Gale)(months 2-3)
c. Optimize reservoir dimensions .....(Gale)(months 3-4)
d. Manufacture and assemble components .....(Gale)(months 4-5)

2. In Vitro NGF release kinetics experiments.....(Gale,Agarwal) (months 5-8)

3. HPLC/ELISA detection of NGF.....(Ambati) (months 5-8)

***Specific Aim 2* -- To evaluate the effectiveness of the conduit-drug delivery device to enhance nerve regeneration across a 15mm nerve gap in a rat sciatic nerve model.**
Tasks/Subtasks:

1. IACUC approval, obtain N=55 animals (48 experimental animals) (We are requesting 7 additional animals from IACUC for possible early losses) .....(Agarwal) (months 8-10)

2. Experimental Groups 1-3 (n=16/group) .....(Agarwal) (months 10-14)
   a. Sacrifice of half animals at day 21 (n=8/group) .....(Agarwal) (months 10-12.5)
   b. HPLC/ELISA for NGF detection of day 21 animals (n=8/group) .....(Ambati) (months 12.5-13.5)
   c. Sacrifice of half animals at day 90 (n=8/group) .....(Agarwal) (months 13-14)
   d. Walking Track(all animals n=16/group).....(Agarwal) (months 10-14)

3. Explanted tissue analysis .....(Agarwal,Ambati) (14-16 months)
   a. HPLC/ELISA for NGF detection of day 90 animals (n=8/group) .....(Ambati) (months 14-15.5)
   b. Nerve histology and IHC .....(Agarwal) (months 14-16)
   c. Muscle Histology .....(Agarwal) (months 14-16)

4. Data analysis and Manuscript Preparation (Agarwal,Gale,Ambati)(months 17-18)

What was accomplished under these goals?

1. Major Activities.

1. Manufacture Devices for use in 15mm nerve gap ..... **(completed)**.
    a. Optimize PGLA ratios
    b. Optimize nanoporous membrane dimensions
    c. Optimize reservoir dimensions
    d. Manufacture and assemble components

2. In Vitro NGF release kinetics experiments ..... **(completed)**.
3. HPLC/ELISA detection of NGF **(completed)**.
4. IACUC approval, obtain N=55 animals(48 experimental animals) **(completed)**.
5. Experimental Groups 1-3 ..... **(To be done)**
    a. Sacrifice of half animals at day 21
    b. HPLC/ELISA for NGF detection of day 21 animals
    c. Sacrifice of half animals at day 90
    d. Walking Track
6. Explanted tissue analysis ..... **(To be done)**
    a. HPLC/ELISA for NGF detection of day 90 animals
    b. Nerve histology and IHC
    c. Muscle Histology
7. Data analysis and Manuscript Preparation..... **(To be done)**

2. Specific Objectives.

1. To optimize release kinetics of NGF *in vitro* using our novel drug delivery conduit
2. To evaluate the effectiveness of the conduit-drug delivery device to enhance nerve regeneration across a 15mm nerve gap in a rat sciatic nerve model.

3. Significant Results

Device Fabrication

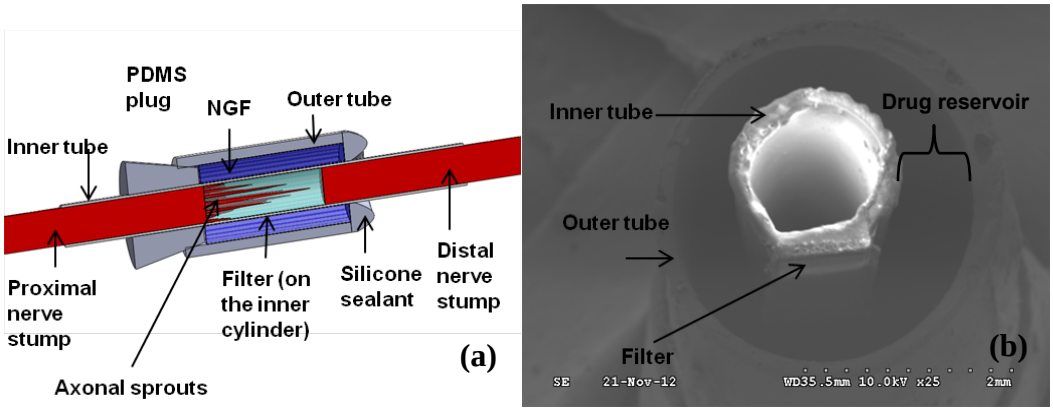


Figure 1 (a) Schematic diagram of a PLGA nerve conduit. Drug (NGF) loaded in the space between the outer and inner tubes will diffuse through the filter and enter the lumen of the inner tube, contacting nerve stumps and stimulating axon growth on the proximal nerve stump. The inner tube can hold the two nerve stumps and guide the new-grown axon to meet the distal nerve stump. Silicone sealant and a PDMS plug are used to seal and connect the two tubes; (b) A scanning electron microscope image of the transverse cross-sectional view of the PLGA nerve conduit. The filter is attached on a window on the inner tube to allow the drug (not shown) stored between the inner tube and the outer tube to release into the lumen of the inner tube and promote local axonal outgrowth on the proximal nerve stumps

We fabricated and tested more than 30 devices. No obvious leakage of the device was noticed when filling the reservoir chambers of the nerve conduit with NGF. However, the drug reservoir volume was observed to be less than designed when filling the device with a calibrated syringe. An average of 25.5µL drug reservoir volume (standard deviation of 5.4µL) was found in the devices and controls used in the release test compared to the designed 34.2µL drug reservoir volume. The loss of volume in the reservoir chamber is likely due to the sterilization process as shrinkage has been shown to occur for PLGA devices.

Dextran Release Results

Devices A-D with four 40µm holes demonstrated a cumulative 3.8-9.0% Dextran release in the one-month period, compared to modeling result showing a 10.2% Dextran release, as shown in . The coefficient of variation for the 4 devices was 0.41 at Day 31, suggesting there is still some manufacturing variability or possibly random interference from air bubbles. Nevertheless, continuous release was observed for all devices with holes (A-D) in the one-month period.

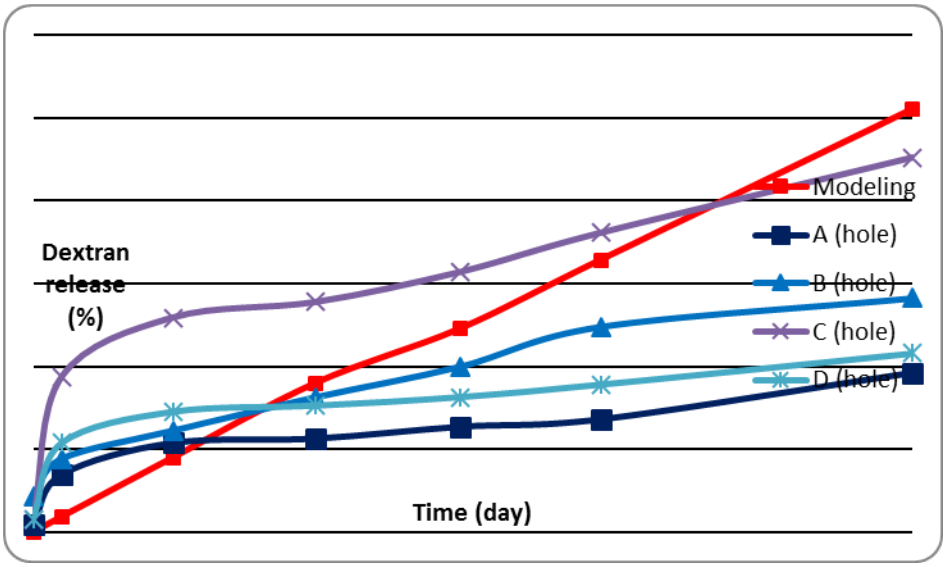


Figure 2 Cumulative Dextran release percentage for the devices with four 40µm holes

The release profile of devices A-D shows a bit of a burst effect, meaning the delivery was relatively high the first day, followed by a fairly steady, nearly zero order, release over the last 4 weeks. Much of the variation associated with device C can be attributed to the variation in burst release on the first day. The cause of the burst release is not clear, but it may be related to small amounts of Dextran being absorbed to the outside of the devices during filling, as in many cases the Dextran leaks out of the device when being filled. The outside of the devices are washed thoroughly, but there may still be some material on the surface. Another possibility is the drug is essentially released somewhat in advance into the inner lumen through the drug delivery holes, as the devices are loaded and then stored briefly. Some drug may be pushed through the drug delivery holes or membranes during loading, or allowed to release through the holes or membranes during storage, and then released quickly once placed in the receiver chambers. In any case, a small burst effect is not necessarily a problem and may be advantageous in initiating the growth of axons in the conduit.

Some of the variation in the release from devices A-D may be associated with the laser drilling process, as the laser drilling process is somewhat challenging to control for these small, rounded

devices. A 5μm measurement error was expected and images of the holes indicates that they have some taper as they cross the wall of the inner conduit, leading to some reduction in diffusion area. Removal of undissolved Dextran might also contribute to the relatively low release profile, when compared to the model predictions, so that less Dextran was filled in the device than designed.

NGF Release Results

Figure 2 shows the cumulative NGF amount released from each PLGA device into the receiver chamber (Petri dish) at a series of time points according to the ELISA readings,. Many of the devices demonstrated a "burst effect" where a large amount of drug was released in the first day before settling into a steady release rate, which will be explored more later. As can been seen in Figure 2, some of the experiments had to be stopped early due to fungal contamination developing in the receiver chamber (the Petri dish) as early as the day 10 collection, and the ones with contamination were discarded without measurement. Therefore, the number of data points for the device samples and controls varied, and only two devices had data for the 25th day collection. None of the controls had data for the 25th day collection.

Since all the media in the receiver chamber was replaced with fresh media during each collection, concentration data measured using the NGF ELISA were converted into NGF mass (ng) and the results were summed over time.

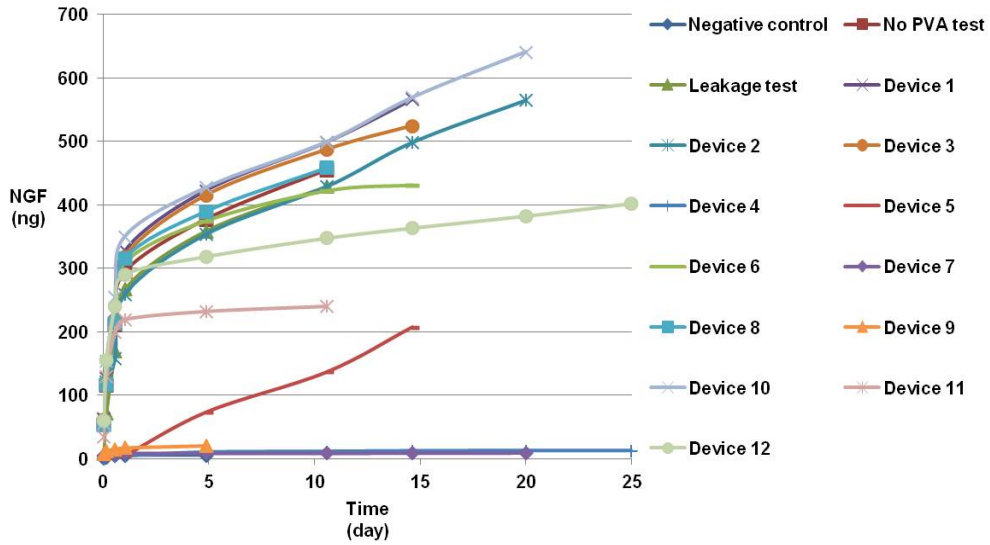


Figure 3 Cumulative NGF amount released into the receiver chamber. In each collection, all the media in the receiver chamber was replaced with fresh media. Thus, the sum of NGF concentration detected in each collection was shown in this figure to present the cumulative amount of NGF released from the PLGA device at each time point. Concentration of NGF and PVA filled in each devices: devices 1-3: 0.1mg/mL NGF in 25mg/mL PVA; devices 4-6: 0.1mg/mL NGF in 12.5mg/mL PVA; devices 7-9: 0.05mg/mL NGF in 25mg/mL PVA; devices 10-12: 0.05mg/mL NGF in 12.5mg/mL PVA

Figure 3 shows the cumulative percentage of NGF released into the receiver chamber at each time point. The cumulative percentage of NGF release was obtained by dividing the cumulative NGF weight by initial NGF weight in the release chamber. The results show that most devices still had more than 50% of the NGF left at the end of the study, and a constant

positive release for all the devices indicates that the PLGA device can continuously supply NGF even after the 25-day period.

Thus, the device as currently designed has the potential to fit clinical applications where a 2 to 3 month consistent release is preferred. The slopes for the data in Figure 3 are different than the ones in Figure 2 because of different volumes and concentrations of NGF – shown in Table 1 – were filled into the devices and controls. NGF daily delivery for the four designs (four combinations of NGF and PVA concentrations) is shown in Figure 4 in which cumulative NGF amount released between each collection

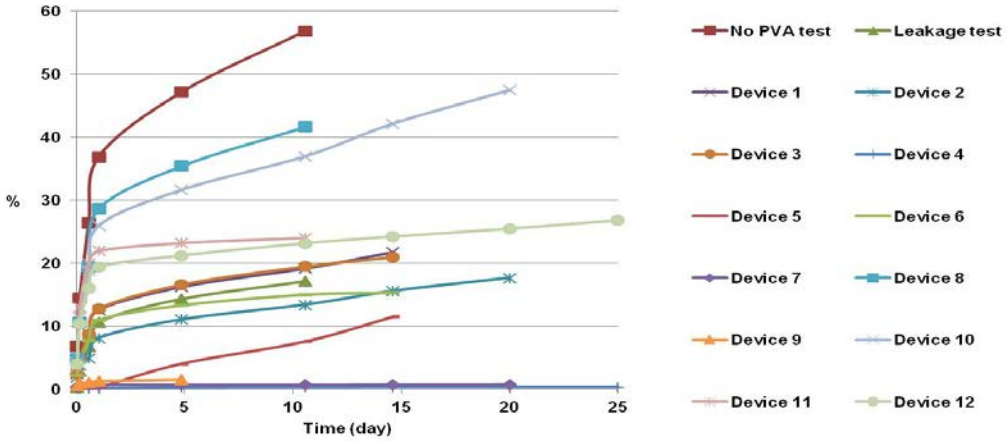
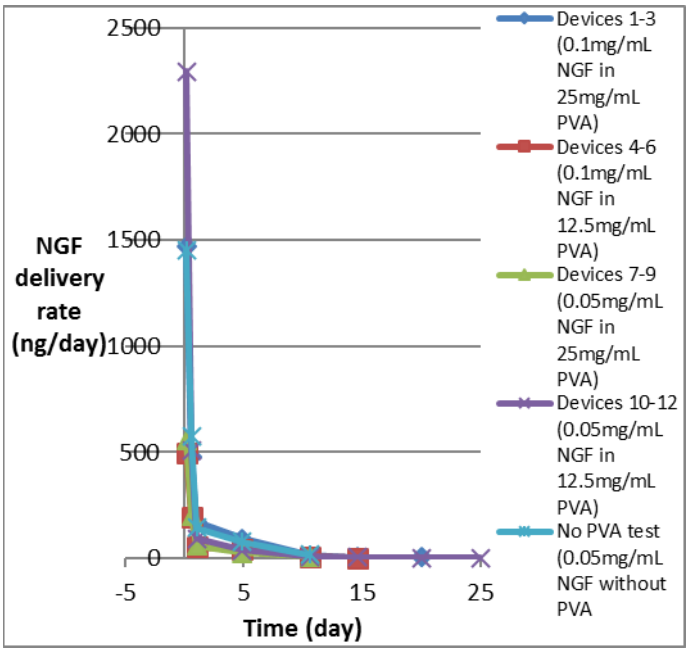
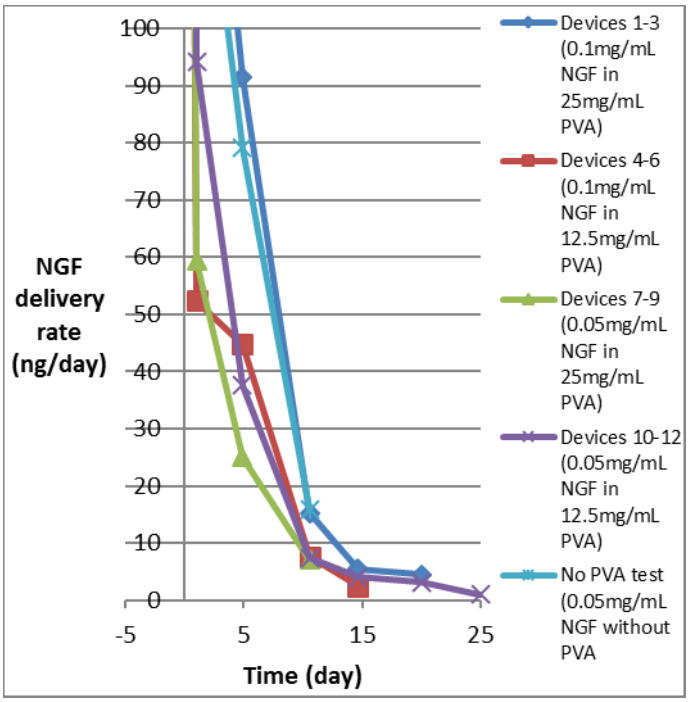


Figure 4 Cumulative percentage of NGF released into the receiver chamber in 25 days



(a)



(b)

Figure 5 NGF release rate comparison. Plotted data is for the average of results for the same design (same concentrations of NGF and PVA). Devices 4, 7 and 9 were excluded from these results due to their extremely low NGF release levels. (a) All data. (b) Zoomed view showing only data below 100 ng/day.

time points were divided by the time period. Devices 4, 7 and 9 were excluded from this figure due to their extremely low NGF release. In Figure 4, devices prepared with the same conditions were averaged.

For devices 1-3 with 0.1mg/mL NGF in 25mg/mL PVA, they showed a similar NGF release in the 15-day period with a cumulative NGF release ranging from 500 to 566ng, as shown in Figure 2. It also shows that a cumulative NGF release percentage of16 to 22 for the 15-day period. They possessed a much higher (1468ng/day) release in the beginning, as shown in Figure 4, then the NGF release rate dropped to 4.5ng/dayin the period between the 15th and the 20th day. Though a higher initial release rate was observed, which is not necessarily detrimental, they showed a release rate higher than the 2ng/day in the later stages of the release test, which was the minimum release rate desired.

Devices 4-6 with 0.1mg/mL NGF and 12.5mg/mL PVA showed a wide range of NGF release percentage and release rate. Device 4 showed almost no release with only 13ng cumulative NGF was released from this device in a 25-day period, while device 5 and device 6 show a 207ng and 431ng cumulative NGF release in the first 15 days, respectively. Due to the low release of device 4, the average daily NGF delivery rate of devices 4-6 is lower than the one of devices 1-3, though devices 4-6 have less PVA and tend to release faster than higher-PVA-concentrated devices 1-3 by design. The most likely cause of the variation among devices 4-6 is the amount and location of the adhesive applied on the filter, which was difficult to control, and the size of diffusion window varied between devices.

For devices 7-9 with 0.05mg/mL NGF and 25mg/mL PVA, they also showed a different NGF amount, release percentage and release rate. Only device 8 continued to release reasonable amount of NGF in the given period, and ended with a 458ng cumulative NGF release in the first 10 days. Devices 7 and 9 have small release, and thus the average of release rate of devices 7-9

is affected to be lower. The reason for the low release of devices 7 and 9 is likely to be the same as device 4, i.e., the filter of devices 7 and 9 were blocked by the adhesive and these devices only deliver less than 21ng NGF cumulatively in the release test. The lower release rate of devices 7-9 (started with 0.05mg/mL NGF in 25mg/mL PVA) compared to devices 1-3 (started with 0.1mg/mL NGF in 25mg/mL PVA) meets the hypothesis that lower given NGF concentration will lead to slower NGF release, although the release rate for the same design (devices 7-9) varied due to diffusion window size difference, which will be improved in the future.

Devices 10-12 with 0.05mg/mL NGF and 12.5mg/mL PVA showed relatively consistent release kinetics. Devices 10-12 possessed the highest NGF delivery rate in the first four hours with the rate of 2295ng/day. The NGF delivery rate dropped to a 3.1ng/day for the period between the 15^{th} and the 20^{th} day, and a delivery rate of 1ng/day in the period between the 20^{th} day and the 25^{th} day, indicating that this combination of NGF and PVA can achieve the desired NGF delivery rate of more than 2ng/day in the first 20-days, while still releasing drug, but at a lower rate, in the following 5-day period.

Other than devices 4, 5, 7 and 9, all devices exhibited a two-step release in which a burst release (average of 286.8ng/day NGF release) was observed in the first day (25 hours), while a slower release was observed for the remaining period. This burst effect might be due to excess NGF that was left on the device when filling, though the devices were washed several times. There is also the potential that during filling, or between the fill time and the beginning of the experiment, that drug was released or flowed into the inner conduit only to then be released when placed in the receiver chamber. This excess NGF was then washed away after replacing the media of the receiver chamber several times.

For the controls, the negative control performed as expected with a very low measure of NGF. For the leakage test, 17.1% of NGF was released from its PLGA device, showing that the device was not totally sealed. Although the release percentage from this leakage test is smaller than half of the devices, it still possesses a “release”, and thus a more careful fabrication process needs to be employed to ensure the sealing of the device. The no PVA test showed the highest NGF release with a 455ng cumulative NGF released into the receiver chamber in a 10-day period. When converting to percentage release, 56.9% of NGF was released into the receiver chamber in the 10-day period, confirming that the absence of PVA would allow for more rapid NGF release.

The hypotheses of different PVA and NGF concentration filled in the device are that high PVA will result in low NGF release, and high NGF will result in high NGF release. Within the same given PVA concentration, devices 1-3 (with 0.1mg/mL NGF) have a higher NGF release rate compared to devices 7-9 (with 0.05mg/mL NGF), which fits the expectation. Devices 4-6 (with 0.1mg/mL NGF) also have a higher NGF release rate compared to devices 10-12 (with 0.05mg/mL NGF), which also fits the expectation. On the other hand, within the same given NGF concentration, devices 1-3 (with 25mg/mL PVA) has higher NGF release rate compared to devices 4-6 (with 12.5mg/mL PVA), which does not fit the expectation. It is due to the extremely low release of devices 4 and 6 affect the average release rate in devices 4-6. For the devices and control filled with the same 0.05mg/mL NGF, no PVA test (with 0mg/mL PVA) has the highest NGF release rate compared to devices 7-9 (with 25mg/mL PVA) and devices 10-12 (with 12.5mg/mL PVA). Devices 10-12 also show a higher NGF release rate compared to devices 7-9. Both of these results match the assumption that PVA will impede and control the NGF release.

Both the positive control and the negative control met expectations and set a 103ng and 6ng cumulative NGF release boundary for the devices; all measurements for devices and controls fell between these values. Though the leakage test did indicate some leakage for that device, some devices (such as devices 4, 7 and 9) showed nearly zero release (but still greater than negative control) and indicate the devices can be sealed effectively- even if that was not the goal for these particular devices. Overall, the results suggest that both the device and the drug concentrations with PVA can be used to release drug in a useful range.

Bioactivity Test in Dorsal Root Ganglion (DRG) cells

Since it was now known that NGF could be released at a desired rate, the next question revolved around the activity of the NGF after being stored in the device and then being released after an extended period of time. The media collected on day 20 from the release tests was delivered to DRG cells to determine if the NGF would still encourage DRG neurite growth.

To provide a reference for these tests, a NGF dosage curve with 0-5ng/mL of NGF on DRG cells, as shown in Figure 5, showed that a maximum average axonal outgrowth of 92µm was reached for these DRG cells when the NGF concentration in the treatment

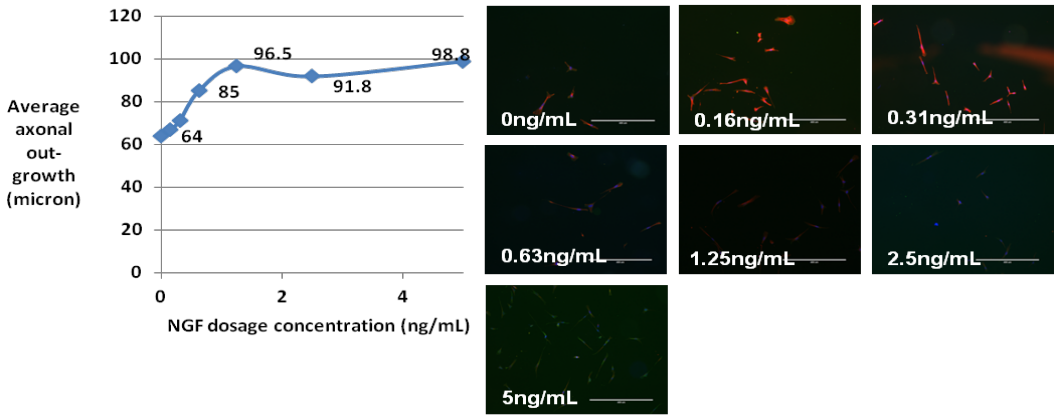


Figure 6 NGF dosage curve on DRG cells. 0-5ng/mL NGF treatments were applied to suspended chick DRG cells for 72 hours to obtain the axonal outgrowth length generated by different NGF concentration. This figure shows that at a 1.25ng/mL NGF concentration, axonal outgrowth reaches a maximum of 96.5µm. In the fluorescence pictures, the purple color represents the cell body of the DRG cells, and the red color represents the axonal outgrowth. The white line is a 400µm scale bar.

was no less than 1.25ng/mL. The NGF released from device 2 and device 10 was chosen for these experiments, as the average 20-day NGF release was more than 2ng/day.

The results for device 2 are shown in Figure 6. Overall, the results showed that the NGF still retained some bioactivity. The 117th-and 480th-hour collections of Device 2 showed an 80.4µm and a76.6µm axonal outgrowth with respect to a 32.3ng/mL and a 23ng/mL NGF concentration, respectively. Both of these concentrations were well above those used for our reference experiments, so direct comparison is not possible, but it is known that there is an optimal NGF concentration, as can be seen in Figure 5, and that excess NGF can lead to slightly reduced outgrowth, as appears to be the case here. In any case, the growth associated with the NGF released from Device 2 is repeatably above the no NGF growth, indicating that the released NGF still has some bioactivity.

Device 10 also showed positive results for NGF bioactivity, as also shown in Figure 6. The treatments from the 117th-and 480th-hour collections showed a 95.7µm and a 89.1µm axonal

outgrowth with respect to a 26.3ng/mL and a 24.8ng/mL NGF concentration. These results again suggest that the NGF released from the device in this period can promote the maximum axon growth in chick DRG cells, and the results from this device are closer to the optimal results obtained from our reference experiments. For the NGF released between the 351st and the 480th hour, it could still result in an average of 89.1µm axonal outgrowth, which demonstrates that the nerve regeneration device is capable of delivering bioactive NGF for the 20-day period.

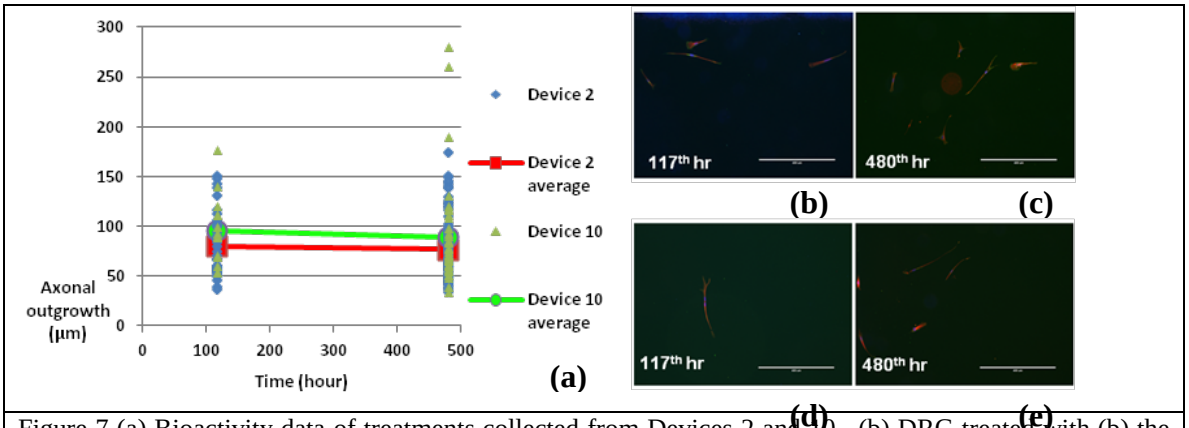


Figure 7 (a) Bioactivity data of treatments collected from Devices 2 and 10. (b) DRG treated with (b) the 117th hour collection from Device 2; (c) the 480th hour collection from Device 2; (d) the 117th collection from Device 10; (e) the 480th hour collection from Device 10. The 117th and 480th hour medium from the receiver chamber of Devices 2 and 10 were applied to chick DRG cells for 72 hours in order to verify the bioactivity of NGF in these treatments. This figure shows that some signals for these treatments, indicating that the NGF in the latter treatments was bioactive to promote axonal outgrowth. The white bar represents 400µm.

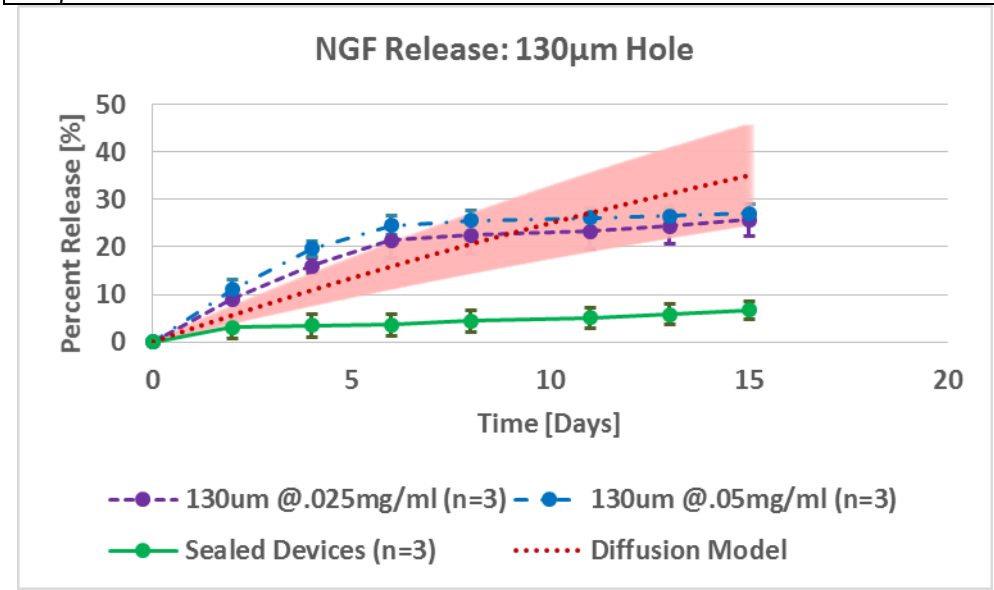


Figure 8 Release data for NGF with single 130 um hole. The device is an all PLGA with inner conduit and PLGA drug reservoir. A CO2 laser is used to create diffusion hole in PLGA conduit. No filter membrane is used for this device thus simplifying manufacturing.

Experimental Parameters

- Single reservoir devices (sterile)

- Target release: ~60% diffusion over 30 days, 2-10ng/ml/day
    - 1x 130μm hole per reservoir
- Loaded drug: NGF
    - ~20μL @.05mg/ml (target: 14ng/ml/day)
    - ~20μL @.05mg/ml (target: 7ng/ml/day)
- Receiver chamber: 3mL media/FBS matrix
    - Changed to PBS after 6 days to minimize contamination effects
- Tested @37°C

Results

- Tests resulted in contamination after 2 days
    - PLGA and NGF have been shown to break down enzymatically and under adverse pH levels
- Diffusion dropped dramatically after 6 days

Figure 8 shows that the diffusion rate is independent of loaded concentration. Additionally, the dosage values can be adjusted linearly.

Conclusion

The proposed PLGA nerve conduit with either 0.1mg/mL NGF in 25mg/mL PVA or 0.05mg/mL NGF in 12.5mg/mL PVA stored in a drug reservoir can constantly deliver bioactive NGF for a 25 day period to the nerve regeneration conduit. The released NGF promoted nearly maximal axonal outgrowth when applied to chick DRG cells. Nine out of 12 tested devices possessed an average NGF delivery rate of more than the goal of 2ng/day. It also showed that every combination of NGF and PVA tested can result in a daily NGF delivery rate of more than 2ng/day for most of the 25-day period tested. Most of the average NGF release rate results from different PVA and NGF combination fit the assumption of higher NGF and lower PVA will lead to a faster NGF release. Several lessons were also learned about the fabrication and testing of the devices. For example, it was learned that the adhesive application process to attach the filters to the inner conduits needs to be improved, as some devices delivered almost no NGF due to diffusion area clogging.

We have determined that laser drilled hole instead of PES filter works better as the diffusion area. In the one-month Dextran release study, all of the controls acted as expected, showing that the release occurs by the desired route – either through the PES filter membrane or four 40μm diffusion holes and the inner conduit, validating the general drug delivery approach. A model based on Fick's First Law of Diffusion was used to predict the release from the various devices and diffusion hole-based PDMS devices (devices A-D) in the Dextran study. The model results were generally in the same range as the experimentally measured values, but there was significant variation both in rate and overall release for the experimental devices, so the appropriateness of the model is only generally confirmed. Nevertheless, the model is likely valuable for designing future diffusion hole sizes and drug dosages to fit various applications.

Work is underway to further optimize the device. We have determined that single 130 μm diffusion hole is ideal for the optimized drug release. No use of filter membrane results in ease

in manufacturing of the device. The release of NGF was found to follow the fickian diffusion model and standard variation for the drug release is well within the dosage range for NGF.

What opportunities for training and professional development has the project provided?

1. **Completion of PhD research project for Keng-Min Lin.**
2. **Continuation of MS research project for Scott Ho.**

How were the results disseminated to communities of interest?

Nothing to report

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.
We are planning to commence the 90 day animal trials by November 15th 2014. We will report the outcomes of animal trials and plans for further developing the device in future.

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?

As part of this work, we have developed a new mathematical model that can be used by researchers to predict, reservoir volume, drug amount, drug concentration, and diffusion hole size. This model will help researchers to avoid costly and time intensive in-vitro trials.

We have developed fabrication and sterilization protocols for completely biodegradable device and tested the efficacy of the device using in-vitro and DRG studies. This data will help researchers/industry to further develop drug delivery efforts in other areas as well.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

A patent is filed for this novel drug delivery device and nerve guide conduit combination. In future, we are planning to obtain translation research award through DOD which will further allow us to commercialization of this research work.

5. **CHANGES/PROBLEMS:** The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Changes in approach and reasons for change

We have determined that the use of semipermeable filter membrane resulted in non-optimal NFG delivery. We used CO2 laser to drill diffusion holes in PLGA inner conduits. The devices made with this approach has resulted in controlled drug delivery. Additionally, the sterilization of PLGA caused challenges as devices buckled and reservoir walls were found to fuse. We have modified the fabrication procedure by providing a small slit that has resulted in intact prototypes after sterad sterilization.

Actual or anticipated problems or delays and actions or plans to resolve them
The failing of device integrity during sterilization procedure resulted in additional studies for PLGA device fabrication and prototype testing. The modified fabrication procedure has resulted in more consistent fabrication. But this unanticipated problem resulted in a delay in

commencement of the animal trials. We will commence the animal trials by November 15th 2014.

Changes that had a significant impact on expenditures

None.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

None.

Significant changes in use or care of human subjects

None.

Significant changes in use or care of vertebrate animals.

No Change.

Significant changes in use of biohazards and/or select agents

No Change.

6. PRODUCTS:

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

Journal publications.

1. Keng-Min Lin, Bruce K. Gale, Himanshu Sant, Jill Shea, Scott Ho and Jay Agarwal. Drug-delivery nerve conduits for peripheral nerve regeneration, Journal of Micromechanics and Microengineering, in preparation, acknowledgement of federal support (yes)
2. Keng-Min Lin, Bruce K. Gale, Himanshu Sant, Srinivas Chennamaneni, Michael Burr and Jay Agarwal. PDMS drug delivery devices: potential application in nerve regeneration, Biomedical Microdevices, in preparation, acknowledgement of federal support (yes)

Books or other non-periodical, one-time publications.

Keng-Min Lin, IMPLANTABLE DEVICES FOR SENSING AND DRUG DELIVERY IN OPHTHALMOLOGY AND RECONSTRUCTIVE SURGERY, Ph. D. Dissertation, Department of Mechanical Engineering, University of Utah, May 2014, acknowledgement of federal support (yes)

Other publications, conference papers, and presentations. .

Scott Ho, Pratima Labroo, Keng-Min Lin, Himanshu Sant, Jill Shea, Jay Agarwal, Bruce Gale, Bioresorbable Multi-Drug Delivery Conduit to Promote Peripheral Nerve Regeneration, in Proceedings of 2014 BMES Annual Meeting, San Antonio, Texas, October 22-25, 2014.

- **Website(s) or other Internet site(s)**
  http://www.mems.utah.edu/publications/
  This website lists the publications and research originating from Co-PI Dr. Gale's lab.

- **Technologies or techniques**

  Fabrication of biodegradable drug delivery prototypes using PLGA. We will publish journal articles to share the device fabrication techniques.

- **Inventions, patent applications, and/or licenses**
  1. SANT HIMANSHU JAYANT, GALE BRUCE KENT, AGARWAL JAYANT P, LIN KENG-MIN, METHODS AND DEVICES FOR CONNECTING NERVES, Last status change:2013-05-10/ Fill date:2012-10-16, WO 2013066619

- **Other Products**
  1. Mathematical model based on Fick's diffusion law
  2. Fabrication of PLGA prototypes
  3. Use of laser to create diffusion hole

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Jayant Agaral
Project Role:	PD/PI
Nearest Person Month Worked:	0.6
Contribution to Project:	Overall management of the project, guidance to students, weekly meetings and report preparation.

Name:	Bala Ambati
Project Role:	Co-I
Nearest Person Month Worked:	0.6
Contribution to Project:	ELISA and HPLC troubleshooting NGF detection.

Name:	Bruce Gale

Project Role: Co-I
Nearest Person Month Worked: 0.6
Contribution to Project: Device manufacturing, weekly meetings.

Name: Jill Shea
Project Role: Faculty
Nearest Person Month Worked: 1.2
Contribution to Project: IRB approvals, DRG studies, ELISA, weekly meetings.

Name: Himanshu Sant
Project Role: Research Faculty
Nearest Person Month Worked: 1.2
Contribution to Project: Device manufacturing and validation, mathematical model, weekly meetings and report preparation.

Name: Keng-Min Lin/Scott Ho
Project Role: Graduate Student
Nearest Person Month Worked: 2.4
Contribution to Project: Device manufacturing and validation, mathematical model, weekly meetings.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report

If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

NO CHANGE

What other organizations were involved as partners?

NONE

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

None.

QUAD CHARTS:

Attached.

9. APPENDICES: Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

Bioresorbable Multi-Drug Delivery Conduit to Promote Peripheral Nerve Regeneration

Scott Ho[1], Keng-Min Lin[1], Dr. Himanshu Sant[1], Dr. Jill Shea[2], Dr. Jay Agarwal[2], Dr. Bruce Gale[1]
Department of Mechanical Engineering, University of Utah[1]
Department of Surgery, University of Utah[2]

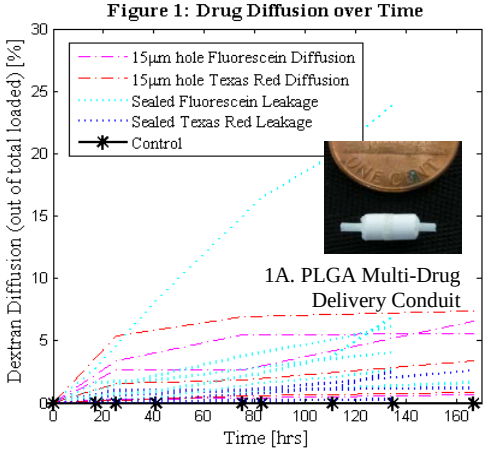
Introduction: Peripheral nerve lesions caused by trauma often require the removal of the injured segment of nerve and subsequent repair by surgery. Synthetic nerve guidance conduits currently are commercially available but they have proven ineffective in promoting sufficient axonal growth. There are major benefits in providing a guidance conduit that can independently deliver multiple localized drugs to the injury site. A bulk diffusion delivery device will provide flexibility in easily alternating drugs as well as precision in using traditional fluid mechanics to control delivery rather than complex polymer degradation. Diffusion kinetics tests were performed to show that this device is capable of releasing drug at a consistent rate over a 30-day period.

Materials and Methods: The bioresorbable guidance conduits were produced using 75/25 poly-lactic-glycolic-acid (PLGA; 7525 DLG 7E, Evonik). The PLGA was dissolved in acetone and ethanol and conduits were then formed and emulsified in water. 15µm diffusion holes were drilled into the inner conduit by pulsing a laser cutter. Final assembly of mold-formed dual conduits and end caps was done using a solvent bonding process, resulting in two ~15µL drug reservoirs.

Two tests have been performed: an initial sealing test and a pilot diffusion kinetics test. Two types of Dextran were used to replicate drug kinetics to test the conduits: Fluorescein (D1821, Molecular Probes; Ex. 494 Em. 521) and Texas Red (D1863, Molecular Probes; Ex. 595 Em. 615). These simulated drugs were loaded into independent conduit reservoirs and then placed into a receiver chamber filled with phosphate buffered saline (PBS). A series of sample collections were taken from the receiver chamber over specified time intervals and the chamber was flushed and filled with fresh PBS each time. Florescence readings were taken using a microplate reader and the data was analyzed using MATLAB software to determine drug release kinetics.

Results and Discussion: Figure 1 shows the results for initial release kinetics testing (device shown in 1A). First, this test was effective in showing independent release of multiple drugs. The results of the sealed leakage tests (n=5) validate sealing techniques for the drug-release reservoirs. Over a 5-day period all but one of the devices maintained a cumulative leakage under 10% of total drug release, with over half of the devices maintaining a cumulative leakage under 3%.

Figure 1: Drug Diffusion over Time



1A. PLGA Multi-Drug Delivery Conduit

The diffusion tests (n=7) indicate that 15µm holes allow for a sustainable drug release for much longer than 30 days. The original target diffusion was ~7% diffusion over a 30-day period in order to maintain ~0^{th} order diffusion kinetics. However, these pilot tests show that some inconsistencies in manufacturing or compounded diffusion error can overwhelm the intended diffusion. In order to optimize drug release, a higher diffusion target (τ=30 days, ~63% release) will be attempted to overcome minor unexpected errors while still maintaining a relatively constant drug release.

Conclusions: Results from initial leakage tests indicate successful manufacturing techniques in sealing the devices. Current diffusion through a 15µm hole shows that this device is currently capable of sustaining drug release for 3+ months. Larger holes and/or an array of holes will be tested to optimize drug release over 30 days. Inconsistencies in device quality and diffusion precision will continue to be improved and following sufficient release kinetics tests, in-vitro testing using known effective growth factors will be performed to explore the biological efficacy of the device.

Acknowledgements: This project has been funded by the Department of Defense Congressionally Directed Medical Research Programs Discovery Award and Idea Development Award. Additional thanks to Jeremy Riley for aid in manufacturing.